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New Light on Growth Cone Navigation

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Growth cones on neuronal process navigate over long distances to their targets in the developing nervous system. New work by Menon et al., 2015 in the current issue of *Developmental Cell* reveals that reversible ubiquitination of the actin filament polymerase called VASP is part of the guidance system.

Assembly of the human nervous system depends on the most complicated navigational challenge in biology. The brain contains about one million miles of neurites, long thin extensions formed by neurons to connect to other neurons, muscles, and other cells. Some of these processes are local, but others are a meter long. No two brains are exactly the same, but the overall pattern of these connections is remarkably similar in each person. Thus, the directions for making these connections are encoded in the genome and work with high fidelity. Neurons form neurites by growing thin extensions from the cell body. At the tip of each neurite, a specialized structure called a growth cone leads the way.

How do growth cones move and navigate? These questions have been approached from inside and outside of the cell. Inside, the issue is the mechanism that produces forces to move the cell. Outside, the unknowns are the nature of the external cues and the cell surface machinery that recognizes these cues. Both are now understood in great detail. In between these two systems are transduction mechanisms that convert the cues into decisions about navigation of the growth cone toward its target, and remarkably little has been discovered about these mechanisms. A new paper from Menon, Boyer et al. (Menon et al., 2015) in the current issue of *Developmental Cell* sheds some welcome light on these murky processes.

Biochemists have identified and characterized proteins that drive protrusion of the leading edge of motile cells. The mechanism depends on polymerization of cytoplasmic actin filaments that push on the inside of the plasma membrane (Pollard and Borisy, 2003). Some growing filaments form bundles called filopodia (or microspikes) perpendicular to the front of the leading edge of the cell, and other filaments assemble a mesh of branched filaments in flat lamellar regions between the bundles (Figure 1). Two families of proteins, formins and Ena/VASP, promote the growth of bundles of actin filaments (Edwards et al., 2014). Growth cones use both filopodia and meshworks of actin filaments to move toward their targets (Dent et al., 2011). Making a very conservative assumption that just 100 actin filaments push in each growth cone, growing the million miles of neurites requires the assembly of about 10^{20} actin molecules. These actin subunits are reused many times through cycles of polymerization at the leading edge and disassembly deeper in the cytoplasm.

In parallel, several decades of research using genetics and other approaches have identified many extracellular guidance cues for growth cones, including molecules diffusing in extracellular spaces and other molecules immobilized on cellular surfaces and in the extracellular matrix (Kolodkin and Tessier-Lavigne, 2011). Other work identified receptors on neurons that bind these extracellular ligands and influence movements of growth cones, in some cases attracting them and in other cases repelling them.

Given a reasonable understanding of the biophysics of growth cone movements and years of work on guidance receptors, one would expect researchers to have found how active receptors are connected to cell movement. For example, a growth cone might respond like a white blood cell or an amoeba chasing down a bacterium—both using conserved signaling pathways to link active cell surface receptors to the proteins that control assembly of actin filaments (Devreotes and Horwitz, 2015). In these and many other examples, the signaling pathway from the receptor to actin assembly involves Rho-family GTPases and polyphosphoinositides. These signaling molecules activate proteins that initiate new actin filaments and stimulate their elongation and turnover. However, the molecular connections in growth cones between guidance receptors and responses of cytoplasmic actin assembly have been unclear for years. This impasse suggested that the mechanisms might have unusual characteristics, calling for new ideas.

Menon et al., 2015 have discovered a new transduction pathway connecting the guidance protein netrin and its receptor DCC to actin polymerization. This system attracts growth cones to sources of netrin. The authors present multiple lines of evidence that the pathway from DCC to actin involves reversible ubiquitination of the actin polymerase VASP. Deciphering this mechanism was tricky, since the positive, attractive signal works by reversing inhibition of VASP by ubiquitin.

The authors were led to this exciting discovery by their previous work showing that an E3 ubiquitin ligase called TRIM9 associates with the cytoplasmic domains of the netrin receptor DCC (Winkle et al., 2014). TRIM9 catalyzes formation of a covalent bond between ubiquitin and a target protein. DCC and TRIM9 concentrate at the tips of filopodia along with VASP, a protein that promotes elongation of actin filaments and protects them from being blocked by capping protein (Dent et al., 2011; Edwards et al., 2014). Thus, DCC, TRIM9, and VASP are all located near the site of actin polymerization, so they are in the right place to transduce

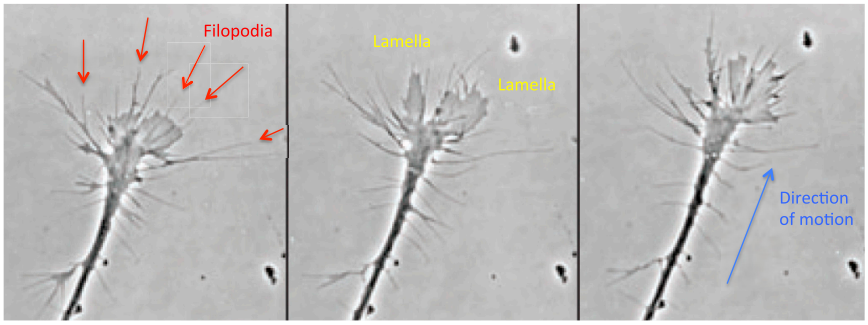

Figure 1. Time Series of Phase-Contrast Micrographs of a Growth Cone of a Cultured Neuron Taken at 1 Min Intervals
The growth cone extends by elongating bundles of actin filaments within narrow processes called filopodia and by filling the space in between with a meshwork of actin filaments. (Work of Dennis Bray, University of Cambridge. Adapted from Pollard and Earnshaw, 2007.)

signals from extracellular netrin locally to cytoplasmic actin machinery.

Menon, Boyer et al. show that TRIM9 is required for growth cones to navigate toward netrin in cell culture and in mouse brains. Furthermore, without extracellular netrin, VASP is ubiquitinated and assembly of actin filaments in filopodia is suppressed. Ubiquitinated VASP is not degraded like many other proteins. Multiple approaches show that VASP ubiquitination is strongly correlated with quiescent filopodia. For example, an inhibitor of enzymes that remove ubiquitin from proteins leaves VASP ubiquitinated and prevents netrin from attracting growth cones.

Adding netrin to quiescent growth cones results in deubiquitination of VASP and growth of filopodia. The authors propose that TRIM9 associated with inactive DCC constitutively ubiquitinates VASP, which turns off the growth of actin filaments locally in filopodia. Furthermore, local stimulation by netrin dissociates TRIM9 from DCC, allowing yet-to-be-identified deubiquitination enzymes to remove ubiquitin from VASP and turning on its actin polymerase activity. All of the evidence is consistent with this proposal. For example, a clever experiment shows that an extracellular gradient of an inhibitor of deubiquitination enzymes repels growth cones, the opposite of attraction to netrin.

The work of Menon et al., 2015 has opened our eyes to a new mechanism controlling axonal guidance, but much more is still to be learned about DCC, TRIM9, and VASP. How does netrin activation of DCC influence its association with TRIM9? How does ubiquitin inactivate the polymerase activity of VASP? Does ubiquitinated VASP cap the ends of actin filaments in filopodia or does it dissociate from the filaments, allowing capping protein or other molecules to limit their growth? How is phosphorylation of VASP related to ubiquitination? What is the structure of VASP, and how does it promote actin polymerization? How do these reactions relate to the actions of formins, a well-characterized family of actin polymerases also found in filopodia? Does ubiquitination mediate the effects of other types of guidance receptors?

Now that growth cone guidance has been shown to involve a novel transduction mechanism, what other surprises will emerge from interrogation of the transduction mechanisms used by other types of guidance receptors?

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